FLOW CYTOMETRY CORE UNIT

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**Titulado Superior* (Advanced Degree)
***Plan de Empleo Joven* (Youth Employment Plan)




OVERVIEW

Flow Cytometry is a fast and multiparametric technology, and an extremely valuable tool in the oncology field. It is an important workhorse able to identify, quantify and isolate defined subpopulations of cells, based on the levels of expression of fluorescent markers and their relation to each other at the single cell level.

Our aim is to provide CNIO Groups with technical and scientific advice regarding the use of cytometric technologies, collaborating with them in the design, acquisition, data analysis, and interpretation of flow cytometry data.

We currently have 4 analysers and 3 high-speed cell sorters with different optical configurations to cater to users' needs. We also have an automated magnetic bead separation system (AutoMACS), 2 automated cell counters (Countess) and a tissue homogenizer (GentleMACS). Analysers are user-operated upon appropriate training, and cell sorters are operated by the Unit staff. Our sorters can separate up to 4- or 6- defined populations simultaneously, as well as perform single cell cloning and index sorting. We can accept human samples to sort under BSL2 regulations.

RESEARCH HIGHLIGHTS

We provide state-of-the-art equipment and software packages in flow cytometry and collaborate with CNIO investigators in setting up and optimising flow cytometry techniques relevant to their research projects. Some of the applications developed and validated by our Unit include:

- → Cell proliferation studies (CFSE, Cell Trace Violet, BrdU or EdU, DNA content, etc.).
- → Apoptosis studies (Annexin V, Mitochondrial Membrane Potential, Caspase 3, etc.).
- → Multicolour Immunophenotyping panels (B and T cell development, Tregs, Inflammation, etc.).
- → Functional assays (side population detection, Ca^{2+} flux, intracellular pH, etc.).
- → Cytometric Bead Arrays to measure several cytokines from cell extracts and plasma.
- → Platelets studies.
- → Extracellular vesicles detection (microvesicles and exosomes).
- → CTC detection and isolation.
- → Single cell sorting for OMICs analysis.

We further optimised our multicolour flow cytometry panels to characterise immune response in various samples from haematopoietic tissues, pancreas, skin, liver, lung, brain, as well as different tumour types. Single cell deposition using index sorting into 96 or 384 PCR plates to perform single OMICs techniques is now part of our routine portfolio. We also improved the characterisation of our instrument performance by creating voltration templates in all our instruments to assess optimal voltage for each detector, and expanded our training capacities with many more workshops and small practical analysis sessions. This provides our users with more tools to successfully perform their flow cytometry experiments. ■

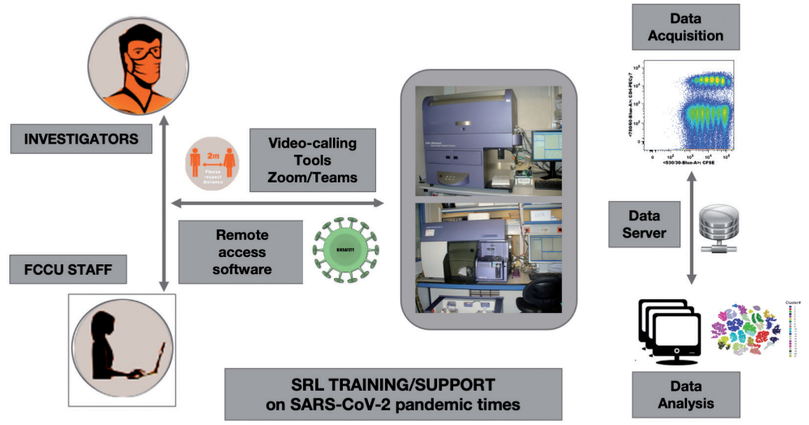

FIGURE At the Flow Cytometry Unit, we put in place a successful workflow for remote training and support in all our analysers and some of our cell sorters so we could continue to work throughout the Covid-19 pandemic, while complying with new social distancing rules.

PUBLICATIONS

- Cerezo-Wallis D, Contreras-Alcalde M, Troulé K, Catena X, Mucientes C, Calvo TG, Cañón E, Tejedo C, Pennacchi PC, Hogan S, Kölblinger P, Tejero H, Chen AX, Ibarz N, Graña-Castro O, Martinez L, Muñoz J, Ortiz-Romero P, Rodriguez-Peralto JL, Gómez-López G, Al-Shahrour F, Rabadán R, Levesque MP, Olmeda D, Soengas MS (2020). Midkine rewires the melanoma microenvironment toward a tolerogenic and immune-resistant state. *Nat Med* 26, 1865-1877.
- Palma F, Affinito A, Nuzzo S, Roscigno G, Scognamiglio I, Infenito F, Martinez L, Franzese M, Zanfardino M, Soricelli A, Fiorelli A, Condorelli G, Quintavalle C (2020). miR-34c-3p targets CDK1 a synthetic lethality partner of KRAS in non-small cell lung cancer. *Cancer Gene Ther*. PMID: 32948832.
- Daniels K, Conway A, Gardner R, Martinez L, Price KM, Schneider S, Sheridan S, Srivastava J, Thornton S (2020). Remote training of SRL users and staff in a global pandemic. *Cytometry A*. PMID: 33175460.
- Aspland AM, Douagi I, Filby A, Jellison ER, Martinez L, Shinko D, Smith AL, Tang VA, Thornton S (2020). Biosafety during a pandemic: shared resource laboratories rise to the challenge. *Cytometry A*. PMID: 33289290.

AWARDS AND RECOGNITION

- Member of the International Society for Advancement of Cytometry (ISAC) Shared Resource Laboratories (SRL) Task Force.
- Treasurer of the European Association "Core Technologies for Life Sciences (CTLS)".